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Influence Of Shipping Container Upon Temperature, Relative Humidity, And Bacterial Growth On Broccoli

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INFLUENCE OF PRODUCE SHIPPING CONTAINER UPON TEMPERATURE, RELATIVE HUMIDIDTY, AND BACTERIAL GROWTH ON BROCCOLI

A Thesis Presented

By

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ABSTRACT

INFLUENCE OF PRODUCE SHIPPING CONTAINER UPON TEMPERATURE, RELATIVE HUMIDIDTY, AND BACTERIAL GROWTH ON BROCCOLI

FEBRUARY 2019

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Directed by Professor Lynne McLandsborough

Temperature and relative humidity of produce throughout the cold chain can greatly affect the quality and safety of the food product. Different packaging systems or containers can provide better cooling environments for food products that could decrease temperature abuse and ultimately safety risks. In this study we compiled temperature and relative humidity profiles of broccoli packed in different shipping containers throughout the produce supply chain. The shipping containers looked at were the wax corrugated box, reusable plastic containers (RPC), and Eco Pack Green Box with and without a lid. Large differences were seen in the temperature profiles of each package during the first 15 hours of the cold chain with the wax-corrugated boxes showing the slowest rate of cooling. Growth rates of *Salmonella sp.* and *Listeria monocytogenes* on broccoli at different temperatures were also determined. *Salmonella sp.* showed a greater ability to grow on inoculated broccoli than *Listeria monocytogenes* during higher temperatures such as 20° C and 37° C. Temperature profiles along with microbial counts from produce lots have been previously recorded; this is the first study to record temperature and relative humidity profiles in conjunction with bacterial growth data of lab inoculated produce.

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CHAPTER 1

INTRODUCTION

1.1 Justification

Fresh produce has been increasing in its production and consumption ever since a large increase in demand during the 1980's, thus causing a larger import from further destinations [1]. The quantities of produce imported into the U.S. have increased greatly over the years. In 1999, 13 million metric tons of fruits and vegetables were imported and grew to 21 million in 2013 [2]. The majority of produce consumed in the U.S. is grown and harvested in Mexico and imported in to the U.S [2] and can take anywhere from 1-6 days to reach its destination. Produce is a highly perishable food that can easily harbor and provide excellent growth conditions for pathogenic and spoilage bacteria. This creates an impending risk involved in eating fresh produce, if it is not handled following proper food safety practices. Each type of fresh produce has an optimal temperature range where it is the least susceptible to bacterial growth, thus optimizing shelf life. If there are breaks in temperature control during distribution from farm to fork, there is potential for greater microbial growth. If the produce is contaminated with a human pathogen, growth of pathogens in the product will contribute to a greater risk of serious foodborne disease.

1.2 Produce Supply Chain

The safety and quality of a produce is almost solely dependent on its time spent in the produce supply chain or the "cold chain". The cold chain is considered the step-by-step process of managing a chilled or frozen food product throughout its

production, distribution, storage and retailing [3]. A common cold chain looks a lot like Figure 1, it starts with harvesting and field packing of produce into containers followed by transportation to an initial distribution center where the produce is palletized and commonly put through a precooling technique. Precooling techniques are commonly used to quickly dissipate the field heat of the produce before cold storage. The shipment of produce is then transported to a secondary distribution center that is more local to its final retail destination, whether that be a supermarket or restaurant. The cold chain ends with the consumer handling and consumption of the produce.

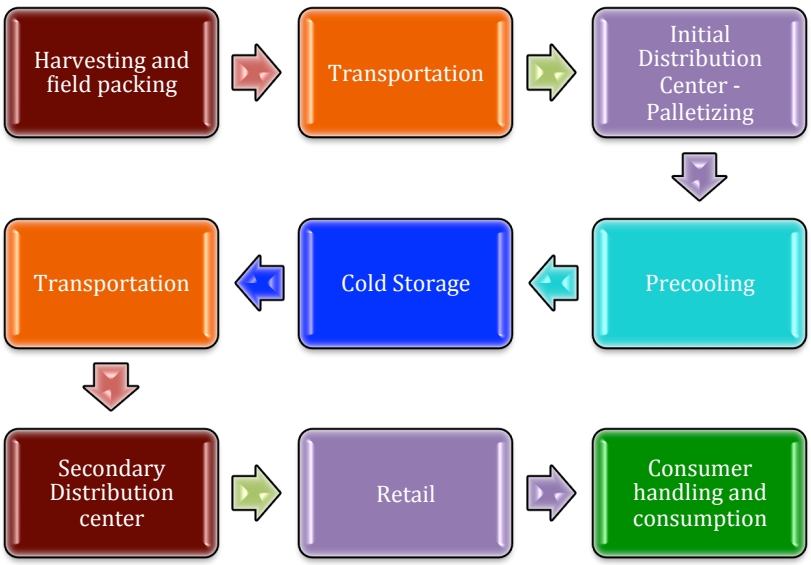


Figure 1: Common Cold Chain

Produce can be contaminated at any point during the cold chain starting with cultivation, where soil microorganisms, feces, animals and insects, and sewage or irrigation water all could potentially cause contamination [4]. A break or mismanagement in the cold chain commonly in the form of temperature abuse increases the risk of spoilage as well as growth of already contaminated produce. However, contamination of produce during postharvest handling most commonly occurs from harvesting and processing equipment, transport containers, and human handling [4]. Practicing proper sanitation procedures and correctly controlling the temperatures of the cold chain is of the utmost importance.

One of the largest concerns in the cold chain is getting the produce to a constant chilling temperature below ambient temperatures, but above -1°C and the time taken to get it there [3]. The longer a product is left at ambient harvest temperature, the higher the risk is of bacterial growth on any contaminated produce. Possible ways to decrease this risk are reducing driving times from the harvest fields to the distribution warehouse and utilizing more efficient precooling techniques to remove field heat. Reducing the driving times is most likely not a viable option so the use of precooling is the best way to increase the rate of cooling.

Pre-cooling is the process of removing the field heat of a produce in order to quickly cool and maintain the safety and quality of the product [5]. Removing the field heat from freshly harvested produce reduces microbial and metabolic activity, respiration rates, ethylene production, water loss, and decreases the ripening rate [5, 6 7]. There are many different techniques used for precooling due to the vastness of different produce and each of their ideal cooling conditions. The main techniques

of precooling produce are hydrocooling, room cooling, forced-air cooling, package icing, and vacuum cooling [5,8]. Some of these techniques are applicable for the cooling of several different products and some products can be cooled by many different methods without a loss in quality or safety but the optimum conditions of cooling are specific for different crops thus demanding a specific precooling technique [9]. The choice of precooling method is influenced by the nature of the product, product packaging requirements, and product flow [5].

Room cooling is the simplest method and is done by setting the containers of produce in a cold storage room after harvesting. It is normally used for products such as potatoes, apples, and pears and other commodities that do not require fast cooling. [8]

Forced-air cooling is the most commonly used method for precooling [10]. It is much faster than room cooling and less expensive than other methods because this method utilizes a fan, canvas sheets, the package ventilation holes and differences in air pressure [10]. The fan creates a higher air pressure on one side of the produce causing the colder air to be forced through the pallet and into direct contact with the warmer product and out the ventilation holes [10]. Forced-air cooling is a fast and efficient cooling method but not as fast as others [10]. Most produce especially in smaller operations can utilize a forced air cooling method some examples are berries, grapes, leafy vegetables, and melons. [8]

Hydrocooling utilizes cold water in either an immersion or shower system where the water directly contacts and cools the product. The water can be mechanically refrigerated or supplemented with ice instead. 100-150 ppm of active

chlorine is commonly added to the water to minimize the spread of postharvest decay and to disinfect the water. Some products such as leafy vegetables and berries cannot be cooled using a hydrocooler due to water-beating damage but produce such as asparagus, broccoli, and artichokes will not be negatively affected and are commonly cooled by hydrocooling [postharvest technology of horticultural crops]. Water removes heat about 15 times faster than air [5].

Ice cooling utilizes direct contact between the product and ice to quickly cool the produce. This requires water tolerant packages and causes the containers to be heavier due to the added ice thus increasing shipping costs. The product is usually one that can tolerate continued exposure to freezing wet conditions such as peas, cantaloupes, sprouts, and broccoli [8].

Vacuum cooling evaporates the water from the product at low atmospheric pressure. The pressure is reduced to the point where the water in the product boils off at a very low temperature causing a more uniform cooling. Vegetables that have high surface-to-mass ratio such as leafy green vegetables, cauliflower, and celery are best suited for this technique [8].

Once harvested, packed, and cooled at the distribution warehouse the produce is then put through any necessary postharvest treatment to ensure its safety and quality before packaging for distribution. After arriving at the initial distribution warehouse and the completion of any precooling or possible postharvest treatments, the produce is stored in cold storage until the designated truck is ready to be loaded and sent out. Cold storage should be 5°C or less to properly limit the growth of any organisms [11]. The refrigerated truck that delivers

the shipment for retail should also be under 5° C. There is no rule in the FDA Food Code on what the relative humidity should be set at but for most produce it should be around 90-100% to decrease water loss and keep the food in good quality [11]. The temperature and relative humidity guidelines should be followed during postharvest storage, shipping, and retail up until consumer application or consumption

1.3 Produce spoilage organisms and pathogen growth

During 1998-2013, there were 972 reported foodborne disease outbreaks in the United States caused by raw produce. These outbreaks resulted in 34,674 foodborne illnesses, 2,315 hospitalizations and 72 deaths [12]. The most common outbreak causing organisms identified were norovirus (54% of outbreaks), *Salmonella enterica* (21%), and shiga toxin-producing *Escherichia coli* (10%) [12]. The proportion of foodborne outbreaks attributed to raw produce to all foodborne outbreaks has been increasing during this time period while the overall number of outbreaks has been decreasing. Meaning, in terms of food safety, raw produce is not increasing its safety as other major food sources are. This is likely because raw produce does have an increased risk to foodborne pathogen growth than most other foods due to its short shelf life.

The most common foodborne pathogens and mycotoxins found on fresh produce are *Salmonella* species, pathogenic *Escherichia coli, Shigella* species, *Yersinia* species, *Listeria monocytogenes, Staphylococcus aureus, Clostridium* species, *Aflatoxin, Ochratoxin A, Citrinin,* and *Patulin* [13]. In 2011 there were two large outbreaks around the world; One caused by *E. coli O104:H4* on Spanish cucumbers

which infected 3842 people throughout multiple countries resulting in 53 deaths and another one caused by *Listeria* in whole cantaloupe grown in Colorado which infected 146 people in the U.S. resulting in 30 deaths [13].

All of the common pathogenic bacteria can contaminate fresh produce during the pre-harvest production phase through various environmental sources or the postharvest preparation phase at both the consumer and commercial levels through cross–contamination of equipment, surfaces, and handlers. *Salmonella* causes contamination through infected fecal matter and can be tolerant to low or high temperatures and extremely acidic environments [13]. *E. coli* causes contamination through infected animal feces primarily that of cattle. It can be tolerant to low pH [18]. *Shigella* causes contamination through human feces and can be tolerant to dried surfaces, low temperatures, and low pH [19]. *Yersinia* is abundant in nature and thus can cause contamination through various environmental sources such as water, soil or insects [20]. *Listeria monocytogenes* is also very abundant in nature causes contamination through soil, decaying vegetation, water, animal feces, sewage, silage, and many other environmental sources. It is tolerant to high salt conditions and low temperatures [22]. *Staphylococus aureus* causes contamination through water and direct contact with human or animal carriers specifically that of their nose, throat, and skin [14]. *Clostridium* species are also common in nature causing contamination through soil, water, and dust [23].

Mycotoxins can contaminate produce during the preharvest phase through seeds, soil, and air or during postharvest storage [14]. If contaminated it is not

possible to eliminate mycotoxins from a food product [15] but they can be partially degraded by physical and chemical methods as well as irradiation [16]. Mycotoxins are generally found growing as fungi on low pH produce such as fruits. Aflatoxin is mostly found in fresh produce such as nuts, figs, dried fruits, and oil seeds. Ochratoxin A if normally found in tropical and sub-tropical produce such as cocoa, coffee, and soybeans but can also be seen in spices, dried fruit, and nuts. Citrinin is commonly found in fruits, herbs, beans, and spices. Patulin is found is fruits such as apples, pears, grapes, bananas, peaches and pineapples. Washing with some sort of a sanitizing agent and sorting in the postharvest phase is commonly all that is done to control the mycotoxin content of fruits [17]. It is clear that the production and preparation phase are the most important in controlling pathogenic and mycotoxin bacterial growth.

1.4 Produce outbreaks

One of the most reported factors in foodborne disease outbreaks where food workers were implicated in its spread was temperature abuse of the food after handling by the infected worker [25]. Even if a food is contaminated one way or another, the spread of a foodborne disease can still be prevented by properly controlling the temperature and sanitization before consumption. Ewen C. D. Todd et al. (2009) looked at 816 foodborne disease outbreaks where food worker errors were implicated. Of the total 1338 errors described for each these outbreaks, 112 of them were related to the temperature of the product at some point throughout the supply chain before consumption (25). The most important survival and

proliferation factors for the pathogen growth in foods was inadequate cold-holding temperatures, allowing foods to remain at room or warm outdoor temperatures for several hours, slow cooling, insufficient time and/or temperature during hot holding, cooking, heat processing, reheating, and insufficient thawing, followed by insufficient cooking [25]. *Salmonella* was the specific bacteria species that was the most associated to errors in cooking/heating time and temperatures. [25].

Relative humidity has also been shown to have an effect on bacterial growth on different produce. However, mismanaging the relative humidity of a produce has not been implicated as a cause of any foodborne disease outbreaks but instead it is often the cause in the decreased overall quality of a produce in the form of water loss. Likotrafiti et al showed that for lettuce leafs, cucumber epidermis, and parsley leaves inoculated with *Listeria* and stored at different temperatures and relative humidity (10°C, 20°C, 30°C, 53% RH, and 90% RH) there was in general slightly more growth of *Listeria* at 90% relative humidity than at 53% [26]. It was shown to be less than a 1 log cfu/g difference in most instances. They concluded that relative humidity had a greater effect on the survival of *Listeria* at cooler temperatures and that while relative humidity at 53% slightly improves the safety of a produce, it can also has a negative effect on the quality of the product [26]. Multiple studies have shown that generally bacterial survival was highest at low and very high relative humidity with an intermediate range around 50% in which was the most deadly to the majority of bacterial species [27].

1.5 Produce Shipping Containers

Reusable plastic containers (RPC) have become commonplace in the produce supply chain due to their high durability and eco friendly nature. They facilitate high rates of cooling due to the many wall openings and open top design, which allow for greater chilling fluid circulation [10, 28, 29]. The openings are evenly distributed on the package walls and bottom inducing uniform cooling during any cooling method [10, 30].

Corrugated boxes are still very commonly used in the produce supply chain due to their low cost and lightweight. They are very versatile and in many instances are made stronger by waxes or other treatments but are then not able to be recycled or reused. The air vents in corrugated boxes decrease their strength and stability thus the vents should be put away from vertical corners and should not account for more than 5% of total box wall area or the boxes will be in jeopardy of collapsing under the weight of the stacked boxes above [10, 8]. This makes it very difficult increase the airflow to the product due to a lack of air vents..

Eco Pack Green Boxes are a relatively new type of shipping container used in the produce supply chain and are utilized in this study. They are similar to RPC containers in that they are have reusable rigid structures as well as recyclable 100% food grade bags which physically hold the produce. They also allow a high amount of airflow to the product with many ventilation holes and an open top design. Eco Pack Green Boxes use 30-70% less raw material than regular wax-corrugated cartons meaning these boxes are lighter and more product can fit on a single pallet increasing profit margins. [31].

CHAPTER 2

OBJECTIVES

The objectives for this study are:

1. Record the temperature and relative humidity profiles of broccoli packed in different types of shipping containers used in the produce supply chain.
2. Determine if the differences in temperature and relative humidity of each package can have a microbial effect on the broccoli.
3. Determine the growth rate of *Salmonella sp.* and *Listeria monocytogenes* on broccoli.

CHAPTER 3

THE INFLUENCE OF PACKING CONTAINER TYPE UPON THE TEMPERATURE AND PERCENT RELATIVE HUMIDITY (%RH) OF FIELD PACKED BROCCOLI DURING DISTRIBUTION FROM FARM FIELD (GUADALAJARA, MEXICO) TO PRODUCE DISTRIBUTIOR (ST. PAUL, MN)

3.1 Introduction

Cooling produce as soon as possible after harvesting and keeping it cool throughout the entire cold chain is of utmost importance in keeping the safety and high quality of the product. The shipping container used for the transportation of produce can have a large effect on the rate of cooling post-harvest and the maintaining of cold temperatures for long periods of time. Ventilated containers for shipping produce need to be designed in a way that the produce is provided a uniform airflow distribution and thus a uniform cooling in order to quickly and effectively cool the product [32]. There is very little data and information on the different produce shipping containers but data is to be collected throughout this study that is important to the comparison of these different containers. The goal of this research was to show differences in the effectiveness of produce cooling in different shipping containers. In order to show this the temperature and relative humidity profiles of broccoli were recorded throughout the cold chain while packed in four types of boxes: wax corrugated boxes, reusable plastic containers (RPC), and Eco Pack green boxes with or without a lid.

3.2 Materials and Methods

3.2.1 Preliminary Logger Experiments

The loggers used to compile the temperature and relative humidity data during this study were the HOBO MX2300 temperature and relative humidity loggers manufactured by ONSET (Bourne, MA). The Loggers have a temperature range of -40 to 70°C with an accuracy of ± 0.25°C from -40 to 0°C and ± 0.2°C from 0 to 70°C. The loggers also have a relative humidity range from 0 to 100% with an accuracy of ±2.5% from 10 to 90% with a maximum of ±3.5% and ±5% below 10% or above 90% relative humidity (33).

Figure 2: HOBO MX2300 Temperature and Relative Humidity Logger manufactured by ONSET

Two of these HOBO MX2300 loggers were purchased and tested initially before the study. For initial testing, the loggers were placed in the different incubators (4, 35, and 55° C) and set to record every 5 minutes to determine variability among different temperature environments. The loggers showed a large variance or interference in the relative humidity while in the large walk-in incubators which we attributed to the compressors of the cooling units. The loggers were placed in a large refrigerated truck similar to that in which the produce is shipped in and this showed much less relative humidity interference than the walk-ins. In order to test their durability and water deterrence of the loggers were set

under a running faucet while logging. One of the loggers died during this test, which prompted the use of a waterproof silicone sealant around the edges of the loggers to provide added protection.

In order to test the loggers' relative humidity accuracy they were placed in two desiccators containing Magnesium Nitrate and Sodium Chloride saturated salt solutions. Magnesium Nitrate has a relative humidity of 58.86% ± 0.43 and sodium chloride is 75.65% ± 0.27 at 5° C. The loggers were placed in the desiccators and let sit over night in the 4° C walk in. This was repeated at room temperature and 37° C. The loggers were also packaged in a car and driven on various bumpy roads to see if the loggers getting knocked around had any effect on the temperature and relative humidity logging.

Once it was decided that the HOBO MX2300 loggers could handle the trip from Mexico to St. Paul, 13 more were ordered and caulked with the waterproofing silicone. These loggers were tested under the same conditions as the first two loggers previously mentioned. A calibrated sensor of the same model and same manufacturing company was also purchased after the study and used to calibrate the other loggers to it. All the loggers were placed in a 4° C refrigerator along with the calibrated one and set to record every minute for about 6 hours. The loggers were also placed in a desiccator with a sodium chloride saturated salt solution in the 4° C walk-in with the calibrated logger.

3.2.2 Preliminary Broccoli Experiments

Broccoli with different consistencies of head/stem was stomached with sterile peptone water in order to find optimal mix for the Mexico and Minneapolis samples. The 3 different consistencies tested were mostly the broccoli head, mostly the stem of the broccoli, and an even mix of broccoli stem and head. 10 g of broccoli was weighed and blended in 90 ml of peptone water in an interscience BagMixer 400 P paddle blender for 4-6 minutes then diluted and plated on TSAYE (.6% Yeast Extract) plates and incubated overnight at 37 °C in order to count colony growth. Two trials were completed to work out methodology for the field study.

3.2.3 Cold Chain monitoring of broccoli transport in different packages.

Temperature and relative humidity loggers were shipped to a collaborator in Mexico. Once recovered, the loggers were added to field packed broccoli during early morning harvest of broccoli at Fortune Growers produce fields in Guadalajara, Mexico. The broccoli was harvested and packed the different packages, and the loggers were attached to the inside of packing crates using zip ties. The four different packaging types used can be seen in Figure 3: Eco Pack Green Boxes with and without a lid, wax corrugated boxes (WCB), and reusable plastic containers (RPC).

Figure 3: Common produce shipping containers utilized in this study.
A - Eco Pack Green Box. B - Wax corrugated box. C - Reusable Plastic Container (RPC). The lid for the Eco Pack Green Box is of similar food grade plastic material as the base bag of the container.

Loggers were added to 4 wax corrugated boxes (the standard packaging), 4 RPC, 3 Eco Pack with a lid, and 4 Eco Pack without a lid. Once packed, all the packages that were going to be on the pallet (with and without the loggers) were stacked onto a truck bed and drove to the distribution warehouse that was about a 2 hour drive away. Once at the warehouse the four package types were stacked on the same pallet with the location of each of the 15 loggers within the pallet recorded to show any variance due to pallet location. There were about 30 total packages stacked 6 rows high on the pallet.

Row	Wax-corrugated	RPC	Eco Pack with Lid	Eco Pack w/o Lid
6	+		+	
5	+	+		+
4		+	+	
3	+			+
2		+	+	+
1	+	+		+

Figure 4: Location of loggers in the stacked pallet of broccoli. The table on the left shows the location of the 15 loggers within the pallet (+), which is pictured on the right just before being put in cold storage after hydrocooling.

After the pallet was assembled at the distribution warehouse, it was put into a hydrocooler (mixture of ice and cold water) for 5 minutes to quickly remove field heat before the pallet was transferred to an on site cooler for holding before shipment. Once removed from the hydrocooler the pallet was briefly unpacked to take microbiological samples then stacked and wrapped as all pallets are and put in the cold storage for about 2 hours until it was loaded on to the refrigerated truck. After being loaded, the packed broccoli was transported to St. Paul MN. The shipment took about 100 hours (4 days) to reach St. Paul including a stop at the Mexico and U.S. border where the containments of the truck were checked. Once the shipment arrived in St. Paul, the pallet was unloaded at a produce distribution center in Minnesota and the data from the loggers was saved onto an Apple Ipad.

3.2.4 Microbiological analysis

Broccoli samples and swab samples of the each package were taken before

and after the shipment. The broccoli samples were cut and put into stomacher bags and sealed. They were cut large enough so that the EIH lab in Mexico could get a useable 10 g piece of broccoli out of it. The broccoli was stomached in a stomacher bag full of 90 ml of sterile Butterfields buffer and diluted and plated in order to conduct the bacterial counts. The swab samples were taken of a 10 cm by 10 cm area of each package type. Swab samples were taken before and after hydrocooling but only one was taken for each package type and due to a lack of samples for either circumstance, it was not enough for statistical analysis. The microbial analysis (standard plate counts, coliform counts, and *E. coli* counts) for both broccoli and swab samples taken in Mexico were performed by EIH Laboratories and Consulting Group (Gajio, Guanajuato, Mexico) utilizing Butterfields buffer as the diluent, and plating on 3M petri film.

In Minnesota, broccoli and swab samples were taken from each package type, and placed on ice. Standard plate counts, coliform counts, and E. *coli* counts were conducted at the University of Minnesota - Food Science Department utilizing Butterfields buffer and 3M petrifilm (plate count and *E. coli*/coliform), within 6 hours of collecting the samples

3.3 Results

3.3.1 Preliminary Logger Experiments

Preliminary experiments were performed to evaluate the suitability of the loggers under a variety of conditions (various temperatures, relative humidity's, and levels of water). During exposure to running water, one of the loggers died, which prompted the use of a waterproof silicone sealant around the edges of the loggers to

provide added protection. None of the loggers that were caulked with waterproofing silicone solution died during any of the preliminary tests. Bumpy road conditions had no effect on the loggers ability to record temperature and relative humidity but the loggers did show a large variance or interference in the relative humidity while in the walk-in incubators.

The initial two loggers tested in the magnesium nitrate and sodium chloride saturated salt solution desiccators showed an average relative humidity of 61.75% and 59.77% for the $MnNO_3$ desiccator and 74.95% and 74.69% for the NaCl desiccator. As said before, magnesium nitrate has a relative humidity of 58.86% +- .43 and sodium chloride is 75.65% +- .27 at 5° C. This shows that either the loggers were slightly off, the loggers have trouble with accuracy at lower relative humidity, or the difference was due to being close to the high disruption from the large compressor in the walk-in coolers.

The data collected from logger calibration was exported to a computer and organized in excel. It showed that the largest average difference in temperature for all the loggers was 0.2° C with the largest single point difference being -0.73° C. The largest average difference in percent relative humidity for all of the loggers was -1.59% with the largest single point difference being 5.79%. The data from each logger during the study was then adjusted accordingly.

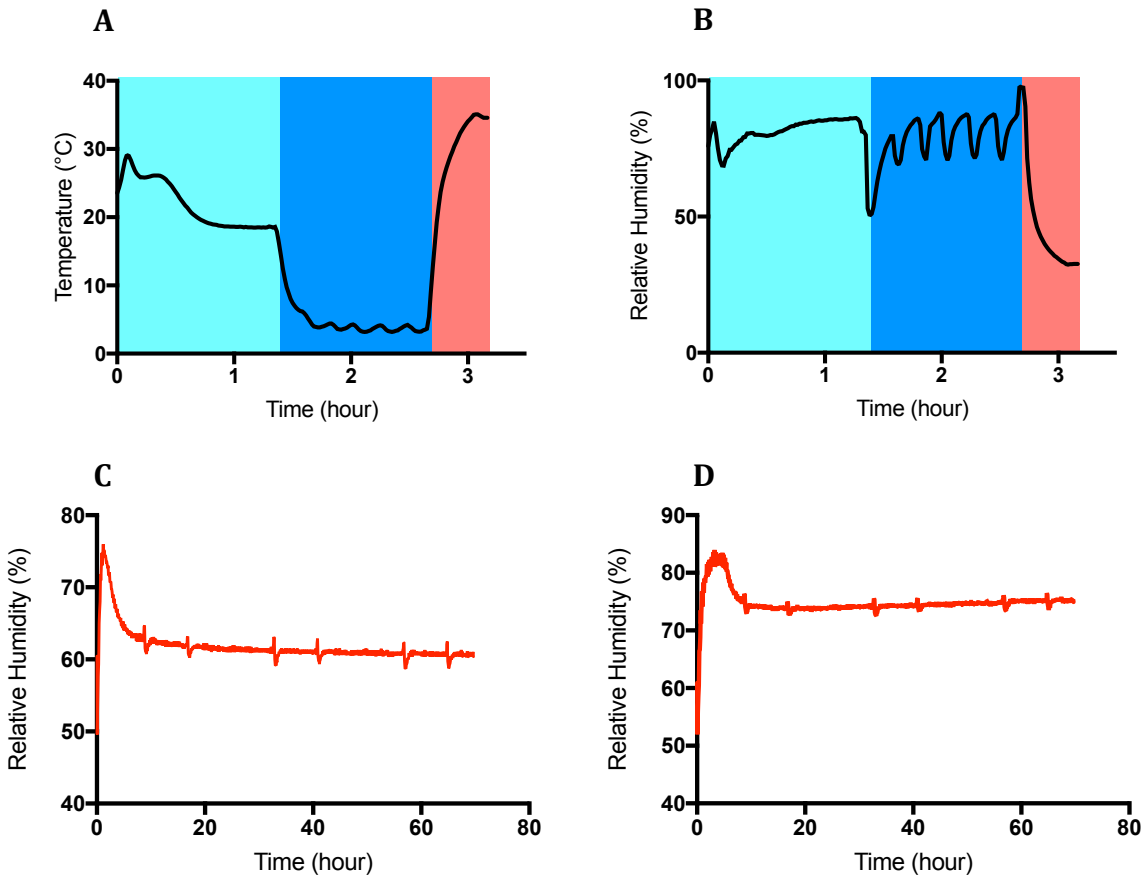


Figure 5: Preliminary logger experiment results: Relative humidity accuracy, waterproof, cooling, and drying experiments.
A) Logger temperature and B) relative humidity during water treatment under a showerhead faucet followed by cooling in a 4° C refrigerator and drying in a 37° C incubator. The light blue portion on the left represents the time spent under the showerhead, the darker blue region in the middle represents the time spent at 4° C and the red region on the right represents the time spent at 37° C.
Graphs C and D represent the Relative humidity data from a logger in the (C) magnesium nitrate (58.86% +- .43 at 5° C) and the (D) sodium chloride (75.65% +- .27 5° C) saturated salt solution desiccators both in the 4° C walk in cooler.

3.3.2 Preliminary Broccoli Experiments

It was found that using mostly the head of the broccoli with little stem stomached and appeared to mix better in the paddle blender than stems alone and was selected for the study. Also, these initial experiments had standard plates counts averaging 3.3 x 10^5 CFU/g (data not shown). The plating scheme in St. Paul was based upon this level.

3.3.3 Results of Cold Chain Monitoring in different packages

The truck containing the pallet of the different packaging types and loggers successfully arrived at the secondary distribution warehouse in St. Paul, Minnesota just short of 4 days. The logger data showed that the rate of cooling was different between different packaging. The hydrocooler blasted the pallet with an ice water and chlorine mix and was very effective at lowering the temperature of the pallet as shown by the loggers but there was also a very noticeable difference in some of the cooling rates, mainly that of the wax-corrugated. The temperature and relative humidity profiles of the first 15 hours of the loggers being active are shown in figure 6 below. These first hours show the greatest variance between the package types.

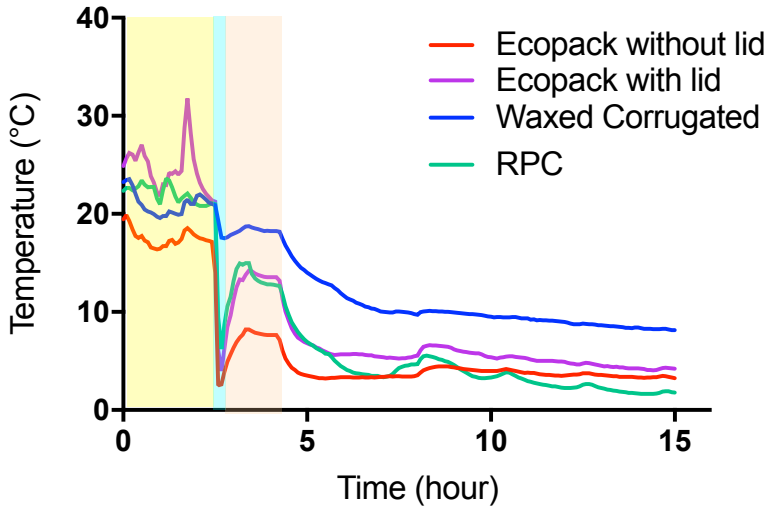


Figure 6: Rate of cooling of each packing type during the first 15 hours postharvest. This graph is the temperature of each packing type during the first 15 hours after harvesting. This outlines the rate of cooling for each container. The yellow shaded region is the time the broccoli and loggers spent traveling from the field to the warehouse. The blue shaded region represents the time the pallet of packaged broccoli spent in the hydrocooler and the tan shaded region represents the time the pallet spent in the on site cooler before being loaded into the shipping truck.

The almost 2.5 hours postharvest and pre-hydrocooling showed differences between the each container type with Eco Pack without a lid showing the lowest temperatures and Eco Pack with a lid at the highest. However, the Eco Pack lids were not added until after arriving at the initial distribution warehouse so the temperature data during pre-hydrocooling for the two Eco Pack boxes should be combined. When combined and averaged during the pre-hydrocooling time period the Eco Pack boxes together averaged 21.1° C, the RPC averaged 22.1° C, and the wax-corrugated averaged 21° C (Figure 6). The variation in temperature during pre-hydrocooling can most likely be attributed to how the boxes were stacked on the truck that transported them to the initial distribution warehouse.

Once inside the hydrocooler the temperature profiles dropped significantly for each package type except the wax-corrugated boxes. During the hydrocooling process the Eco Pack with a lid was cooled from 22° C to 4° C, Eco Pack without a lid from 17.3° C to 2.5° C, RPC from 20.8° C to 6.2° C, and the wax-corrugated from 21.4° C to 17.5° C (Figure 6 and 7).

The slow rate of cooling for the wax-corrugated is likely due to how these boxes are enclosed with not many openings for the ice-cold water to get inside the package to the broccoli. The amount of time taken to reach a steady temperature of <5° C varied by packaging type (Figure 7). It took the Eco Pack without a lid 6.8 ± 3.8 hours to get to <5° C, Eco Pack with a lid took 13 ± 7 hours, RPC took 5.8 ± 0.25 hours, and wax-corrugated 57 ± 7.5 took hours (Figure 6 and 7). The RPC got below 5° C that fastest but was also the only package to arrive in St. Paul with ice still packed inside the container, which is not allowed in the warehouse that it was shipped to and therefor would have been sent back

Package Type	Time till <5°C	Hydrocooling Temp Drop	Avg RH after Hydrocooling
Wax corrugated	57 ± 7.5 hrs	4°C	95.19%
Eco Pack w/ Lid	13 ± 7 hrs	18°C	97.27%
RPC	5.8 ± 0.25 hrs	15°C	92.56%
Eco Pack w/o Lid	6.8 ± 3.8 hrs	15°C	95.33%

Figure 7: Temperature change and relative humidity data of each package type. This table shows the time each package took to get below 5° C, the temp drop during hydrocooling and the average relative humidity after hydrocooling for each container

The relative humidity showed the greatest variance in the hours post-harvest before hydrocooling but does not show a large difference during and after the hydrocooling (figure 8). However, as previously said, the Eco Pack lids were not added until after arriving at the initial distribution warehouse so the relative humidity data during pre-hydrocooling for the two Eco Pack boxes was combined. When combined and averaged the two Eco Pack containers averaged 63% relative humidity pre-hydrocooling while the RPC averaged 63% and the wax corrugated averaged 68%. The variations in relative humidity can be attributed to how the boxes were stacked on the truck bed during initial transportation. During the hydrocooling process the Eco Pack without a lid went from 64% to 94%, Eco Pack with a lid from 70% to 96%, RPC from 69% to 93%, and the wax corrugated from 75% to 87%.

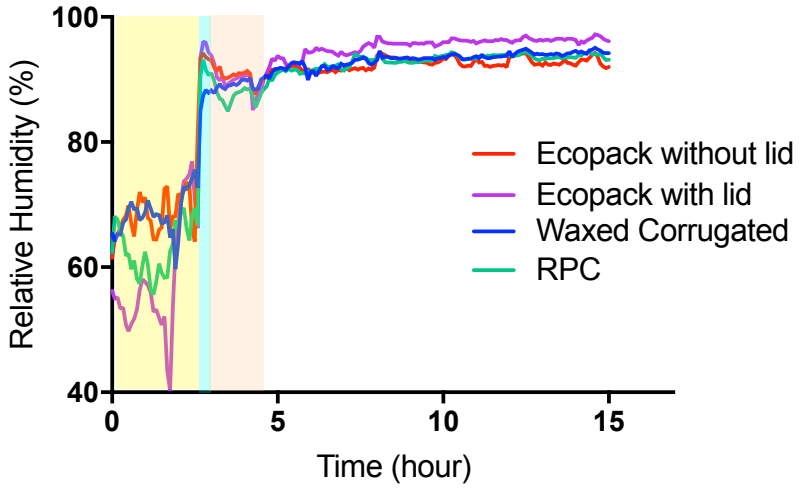


Figure 8: Relative humidity profile of each packing type during first 15 hours postharvest. This graph is the relative humidity (%) of each packing type during the first 15 hours after harvesting The yellow shaded region is the time the broccoli and sensors spent going from the field to the warehouse. The blue shaded region represents the time the pallet of packaged broccoli spent in the hydrocooler and the tan shaded region represents the time the pallet spent in the on site cooler before being loaded into the shipping truck.

Three of the recovered loggers were not operating; we assume it may have been due to water from the hydrocooler breaking the silicone seal. Data from another of the loggers was not used in calculations since it was recovered from a RPC container, which was encased in ice. Overall the useable data came from 3 wax-corrugated boxes, 2 RPC containers, 2 Eco Pack boxes with a lid, and 4 Eco Pack boxes without a lid. The shipment of broccoli was stopped at the borders of the U.S. and Mexico where the doors were opened so they could check the containments of the truck. When they did this a very clear spike is shown on our temperature graphs along with a much less noticeable drop on the relative humidity graphs (Figure 9). It did not take long for the truck to get back to temperature once closed however but it did impede on the cooling of some of the packages especially the wax corrugated. The average temperatures after hydrocooling for Eco Pack w/o lid, Eco Pack w/ lid, wax corrugated, and RPC were 3.28°C, 4.69°C, 5.86°C, and 2.63°C, respectively (Figure 9). The relative humidity was not as variant, but the average humidity after hydrocooling for the RPC was 2.63% lower than wax corrugated while the Eco Pack without a lid and the Eco Pack with a lid were 2.08% and 0.14% higher than the wax corrugated, respectively (Figure 7 and 9).

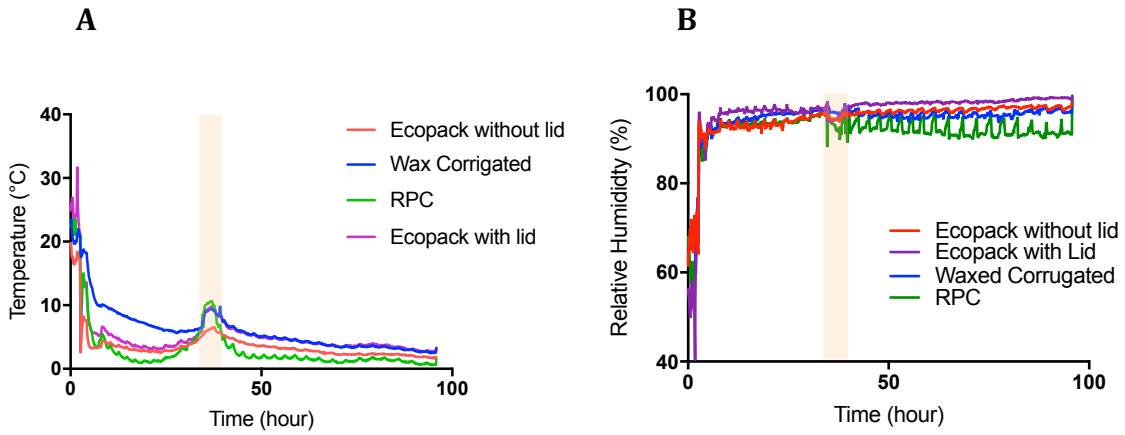


Figure 9: Average temperature and relative humidity of each package type throughout the duration of the shipment.
A) Average temperature profile for each packaging type from field to delivery.
B) Average percent Relative Humidity from field to delivery. The shaded region represents the time when the truck was opened during the border crossing from Mexico to the US.

3.3.4 Microbiological Results

There were not many statistically significant differences in the microbial count data. The standard plate counts and coliform counts of the swab samples did not show a significant difference for any of the packaging types before or after shipping. The highest standard plate count after shipping was seen in the RPC [Figure 11], which was 3.6 ± 0.7 (log CFU/cm^2 ± Std Dev) and the lowest standard plate count was seen in the wax corrugated, which was 2.0 ± 1.3 (log CFU/cm^2 ± Std Dev). No E. coli were detected on any of the surfaces before or after shipping. The broccoli samples also did not show significant differences in standard plate counts, coliform counts, and E. coli counts between packaging types after shipping [Figure 10]. However, the counts were significantly higher for each package type after shipping with the exception of E. coli counts. The broccoli samples shipped in wax-

corrugated boxes showed the highest increase in both standard plate counts and coliform counts though not statistically different from the other packages. The SPC for wax corrugated before shipping was 3.9 ± 0.5 (log CFU/g ± Std Dev) and the coliform count before shipping was 2.6 ±0.8 (log CFU/g ± Std Dev) while the SPC and coliform count after shipping for wax corrugated was 6.1 ± 1.4 and 5.1 ± 1.1 (log CFU/g ± Std Dev), respectively.

Sample	SPC (log CFU/g ± Std Dev)	Coliform count (log CFU/g ± Std Dev)	E. coli count (log CFU/g ± Std Dev)
Broccoli from field	3.9 ± 0.5 [A]	2.6 ±0.8 [A]	1.2± 0.1
Broccoli shipped in Eco Pack with lid	5.5 ± 1.2 [A]	5.0 ± 1.0 [B]	<1*
Broccoli shipped in Eco Pack without lid	5.4 ± 1.0 [A]	4.3 ± 1.2 [B]	<1
Broccoli shipped in RPC	5.0 ± 0.6 [A]	4.0 ± 0.8 [B]	<1
Broccoli shipped in Wax Corrugated	6.1 ± 1.4 [A]	5.1 ± 1.1 [B]	<1

Figure 10: Standard Plate Count (SPC), Coliform count, and E. coli count from the broccoli before and after shipment for each package type. *No *E. coli* were detected on any broccoli samples after shipment. Values in columns with different letters, signify statistically significant differences (P<0.05)

Sample	Package Type (log CFU/cm² ± Std Dev)					
	Eco Pack		RPC		Wax Corrugated	
	SPC	Coliform*	SPC	Coliform*	SPC	Coliform*
Before hydrocooler	2.7	2.2	4	2.3	3.2	2
After hycrocooler	3.4	1.8	3.4	<1	3.6	2
After shipment	3.3 ± 1.3 (no lid)	2.3 ± 0.5 (no lid)	3.6 ± 0.7	1.4 ± 0.2	2.0 ± 1.3	1.3 ± 1.6
	2.6 ± 0.38 (with lid)	2.2 ± 0.1 (with lid)				

Figure 11: Standard plate count (SPC), Coliform count, and E. coli count from swab samples of each package type from before and after shipment. Not enough samples were taken for statistical analysis before shipment. *No *E. coli* were detected on any surfaces at any sampling times and no statistically significant differences were observed.

3.4 Discussion

3.4.1 Efficiency and calibration of HOBO MX2300 Loggers

The temperature and relative humidity loggers showed a susceptibility to water damage, which was combatted by applying a store bought waterproof silicone sealant around the edges of each logger. After this none of the loggers showed signs of water damage prior to the study. The loggers tested in the magnesium nitrate desiccator showed a decrease in accuracy to that of when being tested in a higher relative humidity saturated salt solution such as sodium chloride. One of the two loggers tested in the magnesium nitrate desiccator showed a 2.89% higher relative humidity than what is expected with this saturated salt solution (58.86% +- .43). This could have been due to the logger being slightly off calibration, a decreased accuracy shown when in lower relative humidity, or from the high amount of disruption seen in the relative humidity data of our walk-in coolers. This particular logger showed a higher accuracy with the sodium chloride saturated salt solution (75.65% +- .27) and was on par with the other logger. The sodium chloride

saturated salt solution was used in the calibration step of the other loggers once they arrived. In order to accurately calibrate the loggers the calibration was completed twice at 4° C once just in a closed container and once inside a sodium chloride saturated salt solution. There have been studies done on different technologies used in temperature mapping of produce and the traditional temperature monitoring systems such as the HOBO MX2300 temperature and relative humidity logger produced analogous results with regards in accuracy to that of the RFID temperature tracking systems [34].

3.4.2 Efficacy of Produce Shipping Containers

As previously mentioned, a package that allows uniform airflow distribution will allow a faster more uniform cooling to the product thus, by design, the Eco Pack green box should be able to cool the quickest and most efficient [32]. The Eco Pack green box allows more airflow to the product mainly because the product is free hanging in the package, meaning that the product stacked above is not pressing on the product stacked below thus allowing the airflow above and below the product as well. This is not seen in the RPC and wax corrugated package types. As previously mentioned, the majority of RPC containers arrived at the final destination with ice packed inside which would result in the product being dumped or shipped back ultimately losing yield while the other packages did not show this. The rate of cooling and temperature holding data of the RPC packages cannot be adequately used in comparison to the other package types because of this. The RPC container reached a steady temperature below 5°C one hour faster than the Eco Pack without

a lid but this must be attributed to the ice packed inside the RPC containers. The wax corrugated boxes noticeably showed their inability to allow airflow to the produce and thus had the slowest rate of cooling and only reached a temperature of 17° C during precooling. It took the wax corrugated boxes 57 ± 7.5 hours to reach a steady temperature below 5° C which allows plenty of time for bacterial growth.

3.5 Conclusion

The data presented in this part of the study was gathered directly from the produce supply chain in order to show clear and large differences between shipping containers in the rate of cooling of the produce postharvest. This chapter outlines the methods of testing the equipment to be used as well as the methods of this large-scale international experiment regarding the effects of different package type upon temperature and relative humidity of produce in the cold chain. The efficiency of the HOBO MX2300 loggers was evaluated and the subsequent calibration of the loggers in order to ensure accuracy was outlined. The Eco Pack containers without a lid clearly provided the highest amount of airflow thus causing the rate of cooling during precooling to be highest and allowing it to reach the lowest temperature during precooling of 3° C. Overall this experiment showed that the Eco Pack green box has an inherent ability to cool the produce faster than the other package types without keeping ice stuck in the package. One of the best ways to measure the efficacy of each package type was the time taken to reach a steady temperature below 5° C because this is the time where bacterial growth is most probably on produce during the cold chain. The following chapter will go into detail about the bacterial growth that can occur on broccoli during this time in the cold chain.

CHAPTER 4

LABORATORY BROCCOLI INNOCULATION EXPERIMENTS

4.1 Introduction

As previously mentioned in Chapter 1, temperature abuse of produce in the cold chain can increase the risk of possible growth of harmful bacteria. When handling or preparing food, keeping the food below 5° C and above 57° C is imperative in preventing pathogenic growth [11]. Pathogenic bacteria such as *Salmonella* species and *Listeria monocytogenes* have been shown to survive at temperatures as low as 5° C if the conditions are right [35,36]. *Salmonella enterica* is a gram-negative, facultative anaerobic bacterium that can survive in the environment for extended periods due to its ability to adapt to different temperatures, pH, and water activity than what are normally necessary for growth [36]. Its hosts include cattle, poultry, pigs, insects, and birds that can excrete the bacteria in their feces for months [37, 38, 39]. *Listeria monocytogenes* is a Gram-positive, facultative anaerobic, nonsporeforming, and facultative intracellular bacterium that can cause listeriosis when ingested. The incidence of listeriosis is relatively low but the disease has a high mortality rate in immunocompromised patients commonly exceeding 30%. *Listeria monocytogenes* has a higher survivability in the environment than *Salmonella*; it can multiply at temperatures between 2-4° C, in a pH range from 4.3-9.6, and in the presence of 10-12% sodium chloride and it is able to form biofilms [37, 40, 41, 42]. *Salmonella* was the second most common bacterial culprit of foodborne outbreaks correlating to 34% of the reported cases as reported by the CDC in 2013 [43]. *Listeria* is a much more rare

cause of foodborne outbreaks but it results in much higher hospitalization and death rates [37, 43].

Broccoli is one of the most commonly eaten produce in the U.S. and even though it is not a particularly common cause of foodborne disease outbreaks, has been found to give way to bacterial growth under certain conditions such as controlled atmosphere. There is a lack of data on bacterial growth on raw fresh broccoli, which is a strong reason as to why it was selected for the inoculation and growth of *Listeria monocytogenes* and *Salmonella enterica*.

The goal of the research presented in this chapter is to compile data of bacterial growth on broccoli that can be used to model bacterial growth curves on broccoli in different shipping containers during its time spent in the cold chain. This chapter outlines the methods of the experiments completed during this study regarding the growth of *Listeria monocytogenes* and *Salmonella enterica* inoculated on raw broccoli and grown at temperatures 4° C, 10° C, 20° C, and 37° C and reports their respective results.

4.2 Materials and Methods

4.2.1 Cultures

A five strain cocktail of *Salmonella enteria* was used in this study. The five strain *Salmonella* cocktail consisted of four serovars: (i) Enterititis (strains ATCC BAA 1045 and ATCC BAA 708) originally isolated from almonds and eggs, respectively, (ii) Montevideo (ATCC BAA 710) a human isolate associated with a tomato outbreak, (iii) Gaminara (ATCC BAA 711) isolated from orange juice, and (iv) Michigan (ATCC BAA 709), associated with a cantaloupe outbreak [44] A three

strain cocktail was used for *Listeria monocytogenes* lab strains LM21, LM20, and LM10 are lab strains of *L. monocytogenes* originally obtained from Martin Weidman, at Cornell University and represent lineages I, II and III respectively [45]

Strains were maintained frozen at – 80 °C in TSB containing 25% glycerol. Monthly, each culture was started from frozen stocks into tryptic soy broth with 0.1% yeast extract (TSBYE) and incubated at 37°C for 18h, and then streaked on to tryptic soy agar with 0.1% yeast extract (TSAYE) and incubated at 37°C for 18h to create a working stock. The working stock was kept at 4°C for one month.

4.2.2 Bacterial cocktails

Prior to start of each experiment, each individual strain was grown in TSBYE, after inoculating 1-2 colonies from the working stock and incubated at 37° C for 18 hours. The optical density at 600 nm (OD_{600}) was adjusted to 0.1 from each strain and 1 ml each strain was pelleted and washed twice in 1 ml sterile water, and then suspended in 1 ml water. Washed cells (1 ml) of all five *Salmonella* strains and three *Listeria* strains were combined and the final OD_{600nm} was approximately 0.1. These were the cocktails used to inoculate broccoli.

4.2.3 Broccoli inoculation

Broccoli was bought from the local supermarket and kept in a 4 ° C refrigerator until it was time to inoculate (about 2-3 hours). The broccoli was cut into 25 g pieces ± .5 g of mostly head with little stem, using sterile scalpels and placed in separate stomacher bags creating enough samples for the amount of time points needed for the given experiment. Each broccoli sample was inoculated in a bio safety hood with 5 drops of 5 µl, totaling 25 µl of the cocktail. The inoculum was

given about a 1-hour contact time at room temperature in the biosafety hood or until visibly dry. The stomacher bags (Whirlpak, Nasco, Fort Atkinson, WI) were sealed by rolling and bending of the wire closures. Bags were transferred to incubators at different temperatures (4° C, 10° C, 20° C, and 37° C). To monitor relative humidity in the bags, in each incubator a bag was placed with a 25g piece of un-inoculated broccoli, and a HOBO MX2300 temperature and relative humidity logger.

4.2.4 Monitoring microbial growth in broccoli

Each experiment had an un-inoculated control broccoli as well as an inoculated one representing the 0 time point that was homogenized diluted and plated as soon as the other time points were placed in their respective incubators. The 4°C experiment lasted 21 days and had time points taken at 0, 7, 15, and 21 days. The 10°C experiment lasted about 13 days and had time points taken at 0, 3, 6, 10, and 13 days. There were two 20° C experiments under the same conditions that lasted about 64.5 and 138 hours. The shorter experiment had time points taken at 0, 13, 24, 36.5, 46, and 64.5 hours and the longer one had time points taken at 0, 14.5, 24, 39.5, 66, 91, and 138 hours. There were two 37 °C experiments that lasted about 23.5 and 47 hours. The shorter experiment had time points taken at 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13, 14, 21, and 23.5 hours. The longer experiment had time points taken at 0, 3, 5.75, 9, 11, 13, 20, 22, 24, 27.25, 30, 33, 44.25, and 47 hours.

Once a time point was taken out of its respective temperature incubator, it was diluted with 225 ml of buffered peptone water making a 1/10 dilution, and then stomached until the broccoli was thoroughly broken up (about 4-6 min). From here

the stomached liquid was diluted in buffered peptone water and plated in duplicate for the 20° C and 37° C experiments and triplicate for the 4° C and 10° C experiments, on TSA (for standard plate count), Xylose lysine Deoxycholine agar (XLD, for isolation of *Salmonella*), and Modified Oxford Agar (MOA for isolation of *Listeria*), and incubated at 37 ° C. SPC and XLD were incubated for 24 h, while the Oxford plates were normally let to grow in 37° C for 36-48 hours due to the fact that *Listeria* strains generally to needed more time to grow. The plates were then counted and used to generate growth curve graphs.

An uninoculated 25 g piece of broccoli was also sealed in a plastic stomacher bag with a temperature and relative humidity logger, which was placed at each experimental temperature. The average temperature and relative humidity for each experimental temperature with the broccoli was recorded.

4.3 Results

There were no detectable *Salmonella enterica* or *Listeria monocytogenes* strains on the un-inoculated controls from any of the experiments conducted. Initial cell numbers at T=0 were constantly 4 log CFU/g for the *L. monocytogenes* cocktail, and 4-5 log CFU/g for the *Salmonella* cocktail. The growth of *Listeria monocytogenes* and *Salmonella enterica* as well as a standard plate counts from the inoculated broccoli incubated at each of the four temperatures tested (37° C, 20° C, 10° C, and 4° C) is shown in Figures 12, 13, 14, and 15.

There were high levels of growth variability between trials and even between reps within the same experiment. Two experiments were run under the same conditions as previously mentioned, at both 37° C and 20° C. The 1st

experiment was less than half the length of the 2nd in both instances and thus showed much less growth. Looking at the *Salmonella* graphs (B) in figures 12 and 13 about half the amount growth is seen in the 1st experiment (red) than the 2nd (blue). Only one experiment was run at both 10° C and 4° C with very little growth of *salmonella* at 10° C (Figure 14) and a decrease at 4° C (Figure 15).

Listeria showed similar variability between experiments but the correlation of growth was not as strong as *Salmonella.* Looking at the Listeria graphs (C) in figures 12, 13, 14, and 15, *Listeria* showed much more variance between reps within each experiment at 37° C and 20° C (Figures 12 and 13) but was more consistent at 10° C and 4° C (figures 14 and 15).

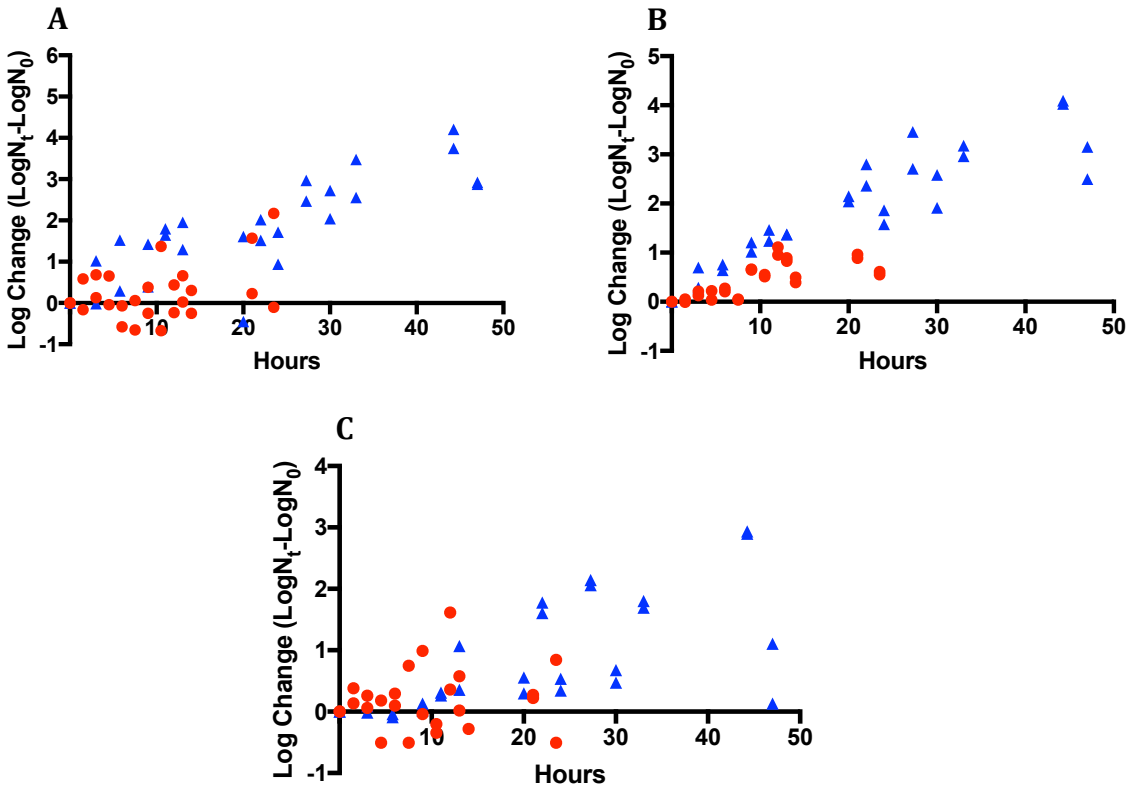


Figure 12: Scatterplot graphs of standard plate count, *Salmonella enterica*, and *Listeria monocytogenes* growth on inoculated broccoli at 37° C over a 24 and 47 hour period. Experiment 1 (24 hours) is shown by red circles (●) and experiment 2 (47 hours) is shown by blue triangles (▲). Graph A is the standard plate count on TSAYE. Graph B is the *Salmonella* growth on XLD agar. Graph C is the *Listeria* growth plated on modified oxford agar. The data is reported in Log 10 CFU/g ($LogN_t$-$LogN_0$). The time points were plated in duplicate so there were 4 total reps for the standard plate count, the Salmonella, and the Listeria growth on broccoli at 37° C.

At 37° C (Figure 12) *Salmonella* grew 3-4 log CFU/g by the end of the 2nd trial (47 hours) while only 1 log of growth was seen by the end of the 1st trial (24 hours). The *Listeria* grew 1-3 log CFU/g at the end of the 2nd trial while 1 log of growth was seen at the end of the 1st trial. The SPC showed growth of 3-4 log CFU/g by the end of the 2nd trial and only about 2 log CFU/g at the end of the 1st with many outliers similar to that of the *Listeria* results. The average temperature and relative humidity during these experiments was 37.2° C and 85%.

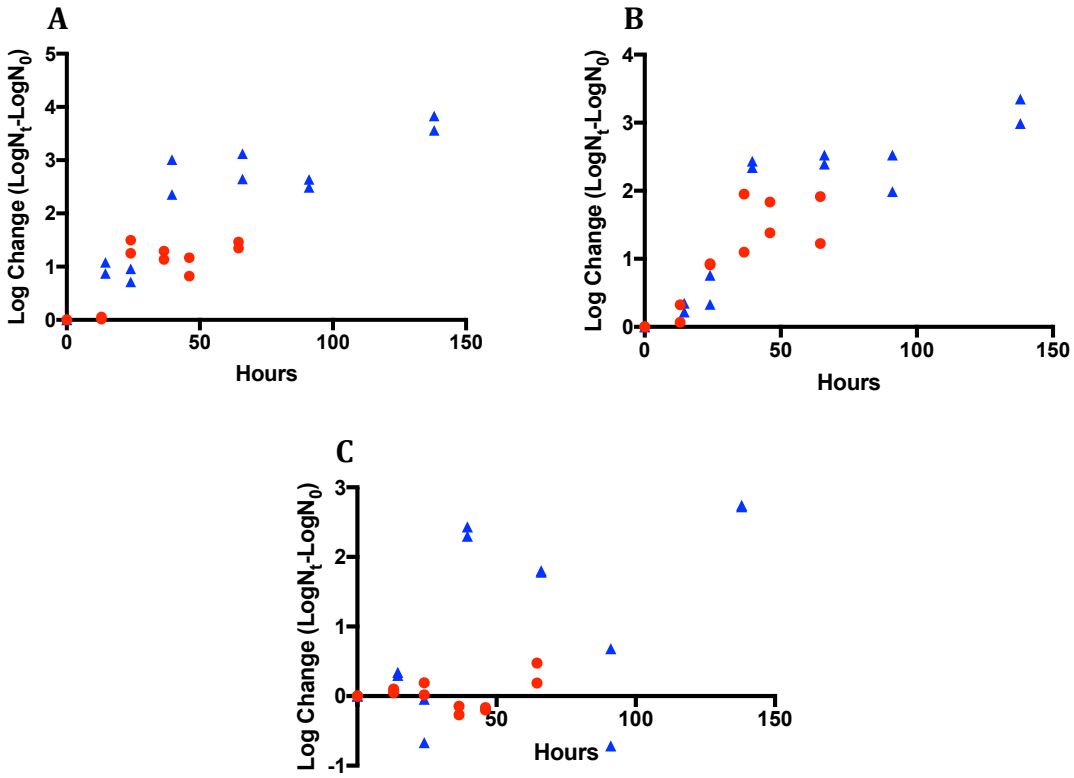


Figure 13: Scatterplot graphs of standard plate count, *Salmonella enterica*, and *Listeria monocytogenes* growth on inoculated broccoli at 20° C over a 64.5 and 138 hour period. Experiment 1 (64.5 hours) is shown by red circles (●) and experiment 2 (138 hours) is shown by blue triangles (▲) . Graph A is the standard plate count on TSAYE. Graph B is the *Salmonella* growth on XLD agar. Graph C is the *Listeria* growth plated on modified oxford agar. The data is reported in Log 10 CFU/g ($LogN_t$-$LogN_0$). The time points were plated in duplicate so there were 4 total reps for the standard plate count, the Salmonella, and the Listeria growth on

At 20° C (Figure 13) *Salmonella* grew over 3 log CFU/g by the end of the 2nd trial (138 hours) and grew just less than 2 log CFU/g by the end of the 1st (64.5 hours). The *Listeria* grew almost 3 log CFU/g at the end of the 2nd trial while barely growing 0.5 log CFU/g at the end of the 1st. The SPC showed growth of 3-4 log CFU/g by the end of the 2nd trial and grew just over 1 log CFU/g at the end of the 1st with a stronger correlation of growth similar to the *Salmonella* results. The average temperature and relative humidity during these experiments was 20.3° C and 89%.

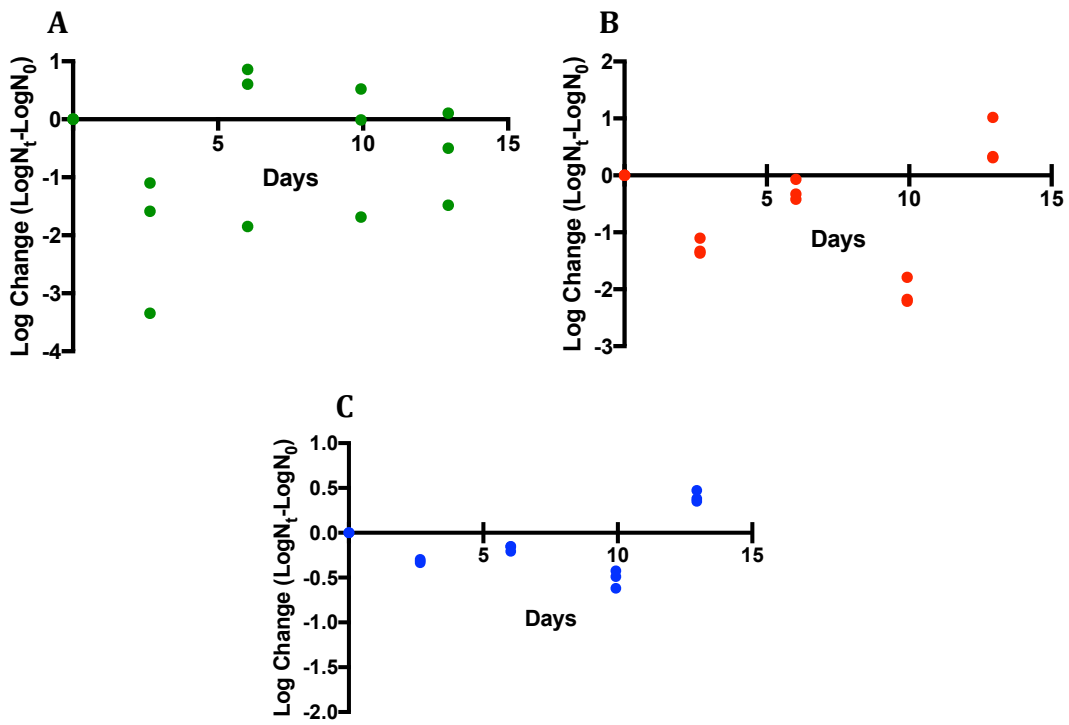


Figure 14: Scatterplot graphs of standard plate count, *Salmonella enterica*, and *Listeria monocytogenes* growth on inoculated broccoli at 10° C over a 13 day time period. Graph A is the standard plate count on TSAYE. Graph B is the *Salmonella* growth on XLD agar. Graph C is the *Listeria* growth plated on modified oxford agar. The data is reported in Log 10 CFU/g ($LogN_t$-$LogN_0$). The time points were plated in triplicate but only one experiment was conducted so there were 3 total reps for the standard plate count, the *Salmonella*, and the *Listeria* growth on broccoli at 10°

After a decrease in growth through 10 days at 10° C, *Salmonella* grew about 1 log CFU/g by the end of the 13 days. The *Listeria* initially decreased 0.5 log CFU/g then ended with 0.5 log CFU/g of growth at the end of the 10° C experiment. The SPC showed a very high amount of variance between the set of triplicate plates. The final counts were about a 1 and 0.5 log loss and one that showed almost no change in growth. The average temperature and relative humidity during these experiments was 11.5° C and 88%. The incubator used during this experiment was accurate to ± 15° F of the room temperature.

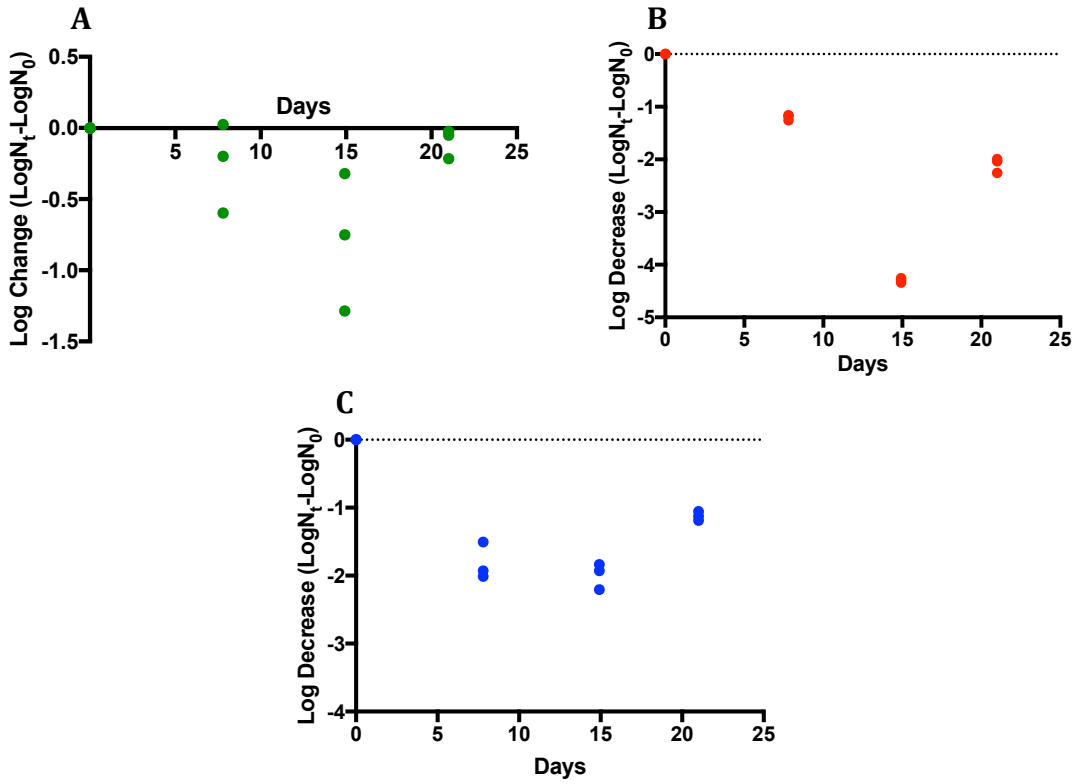


Figure 15: Scatterplot graphs of standard plate count, *Salmonella enterica*, and *Listeria monocytogenes* growth on inoculated broccoli at 4° C over a 21 day time period. Graph A is the standard plate count on TSAYE. Graph B is the *Salmonella* growth on XLD agar. Graph C is the *Listeria* growth plated on modified oxford agar. The data is reported in Log 10 CFU/g ($LogN_t$-$LogN_0$). The time points were plated in triplicate but only one experiment was conducted so there were 3 total reps for the standard plate count, the Salmonella, and the Listeria growth on broccoli at 4° C.

Salmonella decreased 4 log during the 4° C experiment but ended at a 2 log CFU/g decrease after 21 days. The *Listeria* decreased about 2 log CFU/g before ending with about 1 log CFU/g loss. The SPC again showed a high amount of variance between reps similar to that of the 10° C experiment reaching more just more than a 1 log loss at 15 days but ended with almost no change in growth at the end of the experiment. The average temperature and relative humidity during these experiments was 3° C and 88%.

There was another experiment conducted at 20° C which utilized a separate inoculation technique where the *Listeria* and *Salmonella* cocktails were not mixed together and were inoculated on separate pieces of broccoli. This showed about a 1 log CFU/g increase of the initial cell numbers at T=0 but did not provide a difference in growth than what was seen in previous experiments. A graph of the growth during this experiment along with the experiments graphed above can be seen in the supplementary graphs in the Appendix.

4.4 Discussion

The *Listeria monocytogenes* strains (LM 21, 20, and 10) did not show consistent ample growth on broccoli during any of the experiments at 20° C but did show some growth during the two experiments at 37° C. The *Listeria* did however show strong survival at 10° C and 4° C. There may be some inhibitory effects of the broccoli on *Listeria monocytogenes* growth that may be the reason for this decreased growth. The *Salmonella enterica* strains (BAA 1045, 711, 710, 709, and 708) did show consistent growth at 20° C and 37° C. The *Salmonella* showed a higher variability during the 10° C and 4° C experiments than Listeria but ultimately grew more at 10° C.

The choice of broccoli as the growth medium was a decision of logistics and importance. Broccoli may not be a leading produce in connection with foodborne outbreaks but it has still been connected to multiple outbreaks since 1998. With a lack of information on bacterial growth on broccoli and the high popularity of the produce it was a strong candidate for the initial lab trials conducted in this study.

4.5 Conclusion

This chapter outlined the methods used to set up and execute the inoculation of *Salmonella enterica* and *Listeria monocytogenes* on broccoli and the results of its growth or death at various temperatures. The efficiency of growth from both bacterial species was recorded showing that *Salmonella enterica* averaged 1-2 more log growth than *Listeria monocytogenes* at 20° C and 37° C. *Listeria monocytogenes* showed greater survivability at 4° C and more consistent cell numbers at 10° C than *Salmonella enterica*. This data can be further used and applied in order to model growth curves of these bacterium on produce in each of the different packaging that was discussed in chapter 3.

CHAPTER 5

CONCLUSION

Temperature abuse of foods in the produce supply chain or the cold chain can lead to the growth of pathogenic bacterium as well as spoilage and could ultimately increase the spread of a foodborne disease. Increasing the rate of cooling immediately post-harvest is just one of the ways to increase produce safety and quality. The packaging container that the produce is shipped in can have a large effect on the rate of cooling and subsequently the safety and quality of the food.

The Eco Pack green box without a lid showed the fastest cooling rate and got the product to the coldest temperature during hydrocooling without allowing ice to get stuck inside, which decreases the risk of microbial growth the most between the package types. The Eco Pack green box without a lid cooled the product to 2.5° C during hydrocooling while the RPC and wax corrugated cooled to 6° C and 17.5° C. The RPC and Eco Pack containers were very similar in their temperature and relative humidity profiles but the RPC containers arrived with ice still packed inside. The most important data from this part of the study is that of the wax corrugated boxes and its inability to cool the product.

The *Salmonella enterica* and *Listeria monocytogenes* used for laboratory inoculation of broccoli both showed growth on the broccoli at 37° C but the *Listeria* lacked growth at 20° C in the 1st trial while the *Salmonella* showed 1-2 log growth consistently. The *Listeria* showed better survivability than the *Salmonella* at 4° C while showing similar CFU/g at 10° C with less variance.

The research presented throughout this paper was the first to outline the

temperature and relative humidity profiles of broccoli in various shipping containers (Eco Pack w/ lid and w/o lid, RPC, and wax corrugated) in conjunction with laboratory bacterial growth on broccoli at different temperatures. Further research will utilize the temperature and relative humidity profiles of each produce container and the *Salmonella* and *Listeria* growth data on broccoli in order to model bacterial growth curves for different produce in each shipping container throughout the cold chain.

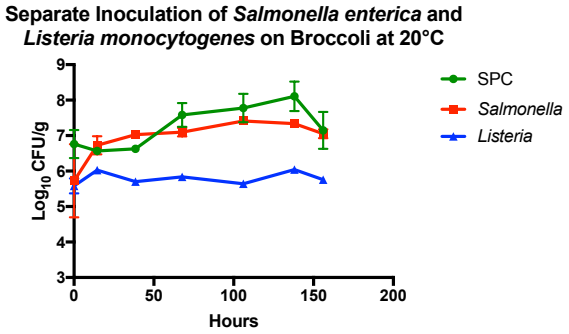
APPENDIX

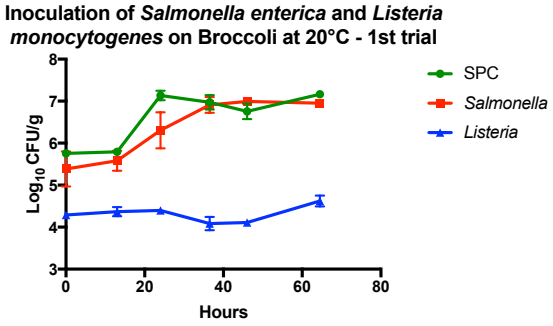
SUPPLEMENTARY MATERIALS

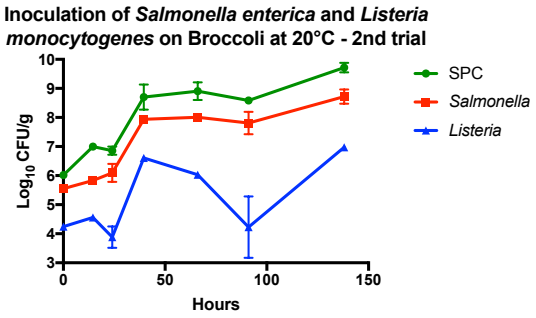
Detailed Culture Preparation

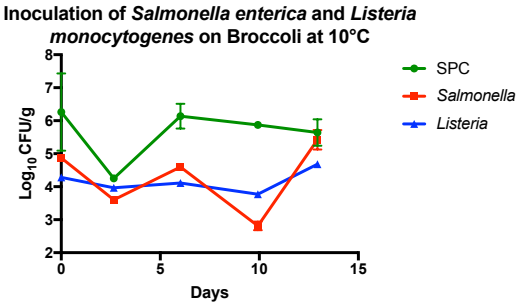
Each bacterial strain was taken from a frozen 50/50 w/v glycerol H_2O bacterial stock solution. 1 glass protect bead of the *Salmonella* strains was removed from the frozen glycerol stock using sterile forceps and placed into 9 ml of triptic soy broth with yeast extract (TSBYE .1%) for each strain and 0.1ml of the *Listeria* frozen glycerol stock was also diluted into 9ml of TSBYE .1% for each strain. All of the *Salmonella and Listeria* TSBYE test tubes were then incubated at 37° C overnight. Once grown the TSBYE was then used to streak plate the bacteria onto their respective selective media plates. Modified oxford agar plates for the 3 *listeria* strains (LM21,20, and 10) and xld agar for the 5 *salmonella* strains (BAA 1045, 711, 710, 709, and 708). These plates grew overnight at 37° C as well and then 1-2 colonies were smeared on a microscope slide to be gram stained and verified through an electron microscope as *Salmonella* or *Listeria* colonies. They were then both streak plated onto triptic soy agar (TSA) plates taking about 5 single colonies from each strain and incubated at 37 ° C overnight and could then be used to start an experiment.

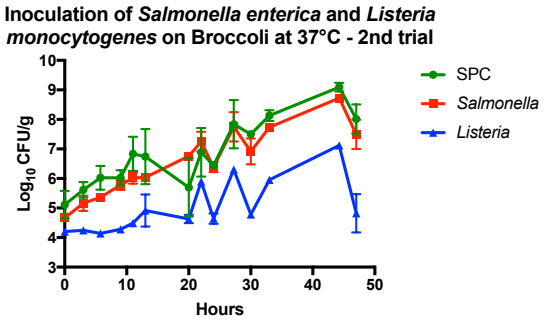
Bacterial Growth Graphs

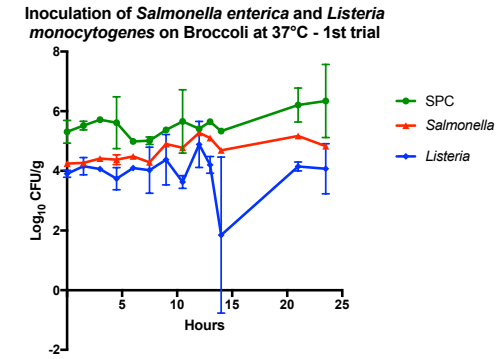


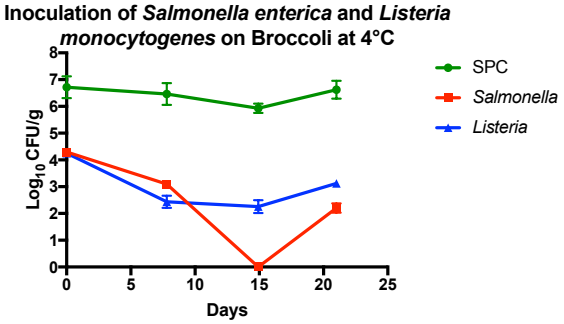












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